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Comparative morphometry and morphology of glochidial shells of Amazonian Hyriidae (Mollusca: Bivalvia: Unionida)

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Abstract: The glochidia of *Diplodon* (*Diplodon*) *suavidicus* (Lea, 1856), *D.* (*D.*) *obsolescens* F. Baker, 1914, *Diplodon* (*Rhipidodonta*) *hylaeus* (d'Orbigny, 1835), *Prisodon obliquus* Schumacher, 1817, *Paxyodon syrmatophorus* (Meuschen, 1781), *Triplodon corrugatus* (Lamarck, 1819, and *Castalia ambigua* Lamarck, 1819 were redescribed based on comparisons of external morphology and morphometric measurements of larval shells. The outline of the glochidial valves was classified into three types. The morphological comparison was based on light and scanning electron microscopy. Details of the shell, hooks and external sculpture allowed a redescription of the larvae, transferring *D. suavidicus* and *D. obsolescens* to the subgenus *Diplodon* and, within the Prisodontini, the genus *Triplodon* Spix, 1827 was separated from *Prisodon* Schumacher, 1817 and *Paxyodon* Schumacher, 1817. The glochidium of *Prisodon obliquus* is described for the first time. The larvae of the latter species and those of *Paxyodon syrmatophorus* present projections on the external sculpture in the form of spikes that have not yet been seen in the larvae of other South American Hyriidae. This unique ornamentation indicates closer proximity of *Prisodon obliquus* to *Paxyodon syrmatophorus*, a result also supported by multivariate analysis that allowed separation of the glochidia of the different species, using morphometric measurements such as length, height, length of the dorsal line and angle of obliquity formed between the position of the base edge in relation to the center of the dorsal line. Considering the angle of obliquity, the glochidium of *Castalia* Lamarck, 1819 was observed to diverge greatly in relation to those of other genera. The sampling dates of adults containing glochidia in the marsupium and an illustrated glossary are provided.

Key words: glochidium, Hyriinae, larvae, size, South America

Resumo. Morfologia e morfometria comparada das conchas dos gloquídios de Hyriidae da Amazônia (Mollusca, Bivalvia, Unionoida).

Os gloquídios das espécies de Hyriidae da bacia amazônica, *Diplodon* (*Diplodon*) *suavidicus* (Lea, 1856), *D.* (*D.*) *obsolescens* F. Baker, 1914, *Diplodon* (*Rhipidodonta*) *hylaeus* (d'Orbigny, 1835), *Prisodon obliquus* Schumacher, 1817, *Paxyodon syrmatophorus* (Meuschen, 1781), *Triplodon corrugatus* (Lamarck, 1819) e *Castalia ambigua* Lamarck, 1819 são comparados morfologicamente e suas medidas discriminadas por meio de análises multivariadas. Os contornos das valvas gloquidianas foram enquadrados em três padrões esquemáticos. A análise morfológica foi realizada com base em microscopia óptica e de varredura. A visualização dos detalhes da concha e dos ganchos permitiu redescrever as larvas, transferir *D. suavidicus* e *D. obsolescens* para o subgênero *Diplodon* e, dentro de Prisodontini, separar *Triplodon* Spix, 1827 de *Prisodon* Schumacher, 1817 e *Paxyodon* Schumacher, 1817. A larva de *Prisodon obliquus* é descrita pela primeira vez. Os gloquídios desta espécie e de *Paxyodon syrmatophorus* apresentaram projeções esculturais externas sob a forma de espinhos não vistas ainda em outras larvas de Hyriidae sul-americanos. Esta ornamentação exclusiva indicou uma maior proximidade de *Prisodon obliquus* e *Paxyodon syrmatophorus*, resultado corroborado pelas medidas dos gloquídios (comprimento, altura, comprimento da linha dorsal e o ângulo de obliqüidade da extremidade ventral em relação ao centro da base dorsal) conforme a análise multivariada. Este método permitiu também observar maior divergência do gloquídio de *Castalia* Lamarck, 1819 em relação às outras espécies analisadas, considerando a variável ângulo. São fornecidos os períodos de coleta dos indivíduos adultos contendo gloquídios nos marsúpios e um glossário ilustrado.

Palavras-Chave: gloquídio, Hyriinae, larvas, tamanho, América do Sul.

The Naiades, freshwater bivalves of the order Unionoida, or Unioniformes (Starobogatov 1991, Bogan and Roe 2008) or Unionida as recently used by Nevesskaja (2009) and Bieler *et al.* (2010), have a larval stage that is usually a parasite of fish (Wächtler *et al.* 2001). Two types of larvae occur, serving as a basis for the division of the Unionoida into two superfamilies (Parodiz and Bonetto 1963): Unionacea [sic] (= Unionoidea) with glochidia and Mutelacea [sic] (= Muteloidea, part of the current Etherioidea) with lasidia. The Unionoida of South America and Australia that belong to the family Hyriidae Swainson, 1840, according to Ortmann (1921), have glochidia that do not possess microstylets on the base and on the surface of the hooks, which are common in the Unionidae, according to Clarke (1981, 1985).

Glochidial shells of South American Hyriidae are variable in length and are between 200 and 350 μm, depending on the species (Ortmann 1921, Bonetto and Ezcurra 1965, Mansur and Campos-Velho 2000, Vale *et al.* 2005). The glochidial shell has two dorsally articulated valves forming a straight dorsal line. The outline of the valves is subtriangular with a slightly protruding base edge (Ortmann 1921, Bonetto 1961b, Mansur 1999, Mansur and Silva 1999). The larval shell is perforated by numerous micropores that are visible both on the internal and external surfaces (Mansur and Campos-Velho 2000). With high magnification, ridges and depressions, which appear to be organized in a regular manner (Mansur and Campos-Velho 2000), may be observed on the external shell surface. Some species of Hyriidae have a hook on the base edge of each glochidial valve whereas others are hookless (Ortmann 1921, Bonetto 1961b, Parodiz and Bonetto 1963, Mansur and Silva 1999). The hook is present in species with parasitic glochidia (Bonetto 1955) and species of *Diplodon* (*Rhipidodonta*) Mörch, 1853 are hookless (Bonetto 1967). The shape of the hook may be a simple triangle, as in *Castalia* Lamarck, 1819 (Vale *et al.* 2005), or elongated in the form of an "S" with sharp terminal cusps, as in *Triplodon* Spix, 1827 and *Diplodon* (*Diplodon*) Spix, 1827 (Bonetto and Ezcurra 1963, Mansur and Silva 1999). Internally, the glochidia have a very simple arrangement with a single central adductor muscle, phagocytic cells, sensitive cirri, a posterior ciliary organ, and sometimes, a flagellum (Mansur and Campos-Velho 1990, Wächtler *et al.* 2001).

Studying the Amazonian species of Hyriidae, Ortmann (1921) was the first to use glochidia to recognize species, describing the glochidia of *Diplodon hasemani* Ortmann, 1921 [currently *D. hylaeus* (d'Orbigny, 1835), according to Bonetto 1967] and *Castalia acuticosta* Hupé, 1857 [currently *C. ambigua* Lamarck, 1819, according to Bonetto 1967]. Bonetto (1961b) described and figured the outlines of glochidia of 33 species of *Diplodon*, three of which were from the Amazon basin: *Diplodon hartwrighti* (Ihering, 1910), *D. suavidicus* (Lea, 1856) and *D. hasemani*. Later, Bonetto and Ezcurra (1963) described for the first time the glochidium of *Triplodon corrugatus* (Lamarck, 1819), including this species in the genus *Prisodon* Schumacher, 1817. Bonetto (1965), in a revision of the glochidia of the tribe Castaliini, defined the short triangular hook with its sharp distal extremity and, without lateral projections, as an important diagnostic character of the tribe. More recently, Vale *et al.* (2004, 2005) described and quantified variation in the morphology of the valves of glochidia of *Castalia ambigua ambigua* from the Irituia River, Pará. Using scanning electron microscopy, the authors observed for the first time, the presence of a protuberance on the ventral base of the glochidial hook. Beasley *et al.* (2005) determined the brood size and larval length during the reproductive season (February to September) of *Paxyodon syrmatophorus* (Meuschen, 1781) from the Tocantins River. From the Orinoco basin in Venezuela, the glochidia of *Castalia ambigua multisulcata* Hupé, 1857 (synonym of *Castalia orinocensis* Morrison, 1943, according to Mansur (1991) were described by Martínez (1983) and *Diplodon* (*D.*) *granosus granosus* (Bruguière, 1792) described by Martínez and Royero (1995). Specimens of the latter species have been collected from the Amazon basin (*pers. obs.*).

According to Bonetto (1961b), the morphological characteristics of glochidia are not relevant for distinguishing between species, regardless of being parasitic or not. However, the morphology of the glochidium may have potential as a taxonomic tool, at both species (Mansur and Campos-Velho 1990, Jupiter and Byrne 1997, Mansur 1999) and higher taxon levels (Bonetto 1960, 1961b, Bonetto and Ezcurra 1965, Graf 2000, Sayenko *et al.* 2005). Parada *et al.* (1989) emphasized the importance of morphology and morphometrics of the glochidial shell, as well as the shape and structure of the hooks as distinguishing characters. Greater attention is being given to glochidial morphology, especially fine structure in taxonomy (Wu *et al.* 1999). The present study aims to describe the larvae of the poorly-known hyriid fauna from the Amazon basin by means of a morphological and a morphometric comparison of the glochidia of seven species and five genera as a contribution to the taxonomy of the family.

MATERIAL AND METHODS

Gravid females (with larvae on the external demibranch of the marsupia) were collected by hand from shallow banks and margins of rivers of the Brazilian part of the Amazon. Sampling was carried out during or at the end of the dry season, when the animals are closer to the water's edge and manual sampling is feasible. After capture, the bivalves were maintained for 24 h in river water to which were added crystals of pure menthol ($C_{10}H_{20}O$), in order to relax the animals. Afterwards, the specimens were preserved in 70% ethanol. In some cases, the larvae were removed before fixation and maintained in distilled water for up to five days, in order to macerate the soft parts in preparation for scanning electron microscopy (SEM).

The following Hyriidae that occur in the Amazon basin were included in the analysis: *Diplodon*, *Triplodon*, *Paxyodon* Schumacher, 1817, *Prisodon*, and *Castalia*. Both subgenera of *Diplodon*, *Diplodon* (*Diplodon*) with the glochidial hook in the form of an "S" and with a protuberance at the base of the hook, and *Diplodon* (*Rhipidodonta*), with a hookless glochidium without a protuberance, were also included. Some specimens of *D. suavidicus*, from the Jamari River (Rondonia State), were deposited in the Mollusc Collection at the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus. Specimens of other species were deposited at INPA and in the Laboratório de Moluscos, Universidade Federal do Pará, Campus de Bragança. Larvae from adults that had been fixed in 70% ethanol were removed from the marsupia and hydrated in a series of solutions of ethanol of increasing water content, ending in distilled water. Soft parts were removed with a solution of sodium hypochlorite (four drops of NaClO in 10 ml of water). The cleaning process was monitored under the stereoscope. Once the tissues had been removed, the valves were rinsed four times in distilled water to eliminate the sodium hypochlorite. Afterwards, the material was dehydrated, as described by Mansur and Campos-Velho (1990), in order to mount permanent light microscope slides as well as stubs for SEM and photography.

To avoid crushing the glochidia with the weight of the coverslip during the mounting process of the permanent slides, each group of larvae was surrounded by a single layer of fine strips of paper before sealing the coverslip to the slide with Entellan®. Glochidia were photographed using an Olympus® light microscope digital photography system. The free software CombineZM (2009) was used to obtain focal integration for some figures. A Philips® XL30 and a JEOL® JSM6060 electron microscopes were used to carry out scanning electron photomicroscopy.

To ease the redescription and comparison of glochidia of the species studied, the outlines of the larvae were classified according to three schemes based on the type of triangle: equilateral, isosceles and scalene (Fig. 1). The definition of the anterior and posterior regions of the glochidial valves follows that of Hoggarth (1987). The term spike was based on Callil and Mansur (2005).

Larval shells that were in a maximal horizontal position were drawn and measured under the light microscope for length, height, and dorsal line length (in μm) and the angle of obliquity (measured in °). The angle of obliquity is defined as the angle of the ventral point in relation to the center of the dorsal line (Mansur and Campos-Velho 1990), i.e. the angle between the line that joins the ventral point to the middle of the hinge and the line that joins the ventral point perpendicular to the hinge.

The terminology referring to the glochidial hook was standardized and defined in a glossary (Table 1). The data and illustrations of Vale *et al.* (2005) were added to the description of the glochidium of *Castalia ambigua*.

Variability in glochidium length, height, dorsal line length and the angle of obliquity were analyzed using canonical discriminant analysis (CDA). Groups of larvae are distinguished by CDA which aims to remove the influence of size to allow discrimination among shapes. The multivariate analysis was carried out on natural log transformed data using SPSS 13.0.

Material examined: *Diplodon* (*Diplodon*) *suavidicus*. BRAZIL, **Amazonas:** Novo Aripuanã (Aripuanã River, downstream of Pernambuco Beach 06°01′05.1″S 60°11′39.5″W), 1 specimen, 06.IX.2007, D. M. Pimpão *leg.* (INPA 1273); (Pernambuco beach 06°01′05.1″S 60°11′39.5″W), 1 specimen, 05.IX.2007, D. M Pimpão *et al. leg.* (INPA 1260); 10 specimens, 06.IX.2007, D. M Pimpão *et al. leg.* (INPA 1265); (Aripuanã River 06°01′29.3″S 60°11′35.8″W), 1 specimen, 13.IX.2004, D. M Pimpão and C. Sotero *leg.* (INPA 326); **Pará**: (Xingú River, Neusoranga Beach, 03°27′56″S 51°57′31″W, 1 specimen, 25. XI.2000, C. R. Beasley col. (INPA 1585); **Rondônia**: (Jamari River, Usina Hidrelétrica Samuel, 08°44′S 63°27′W), 1 specimen, 21.VIII.1987, C. S. Motta *et al. leg.* (INPA 205).

Diplodon (*Diplodon*) *obsolescens*. BRAZIL, **Mato Grosso**: Aripuanã, Aripuanã River, upstream of the Dardanelos waterfall, 10°09′49.7″S 59°27′45.9″W, 31 specimens, 06.VII.2007, D. M Pimpão *et al. leg.* (INPA 1235).

Diplodon (*Rhipidodonta*) *hylaeus*. BRAZIL, **Mato Grosso**: Aripuanã (Aripuanã River, upstream of the Dardanelos waterfall, 10°10′08.6″S 59°27′55.9″W) 6 specimens, 05.VII.2007, D. M Pimpão *et al. leg.* (INPA 1230); 2 specimens, 09.VII.2007, D. M Pimpão *et al. leg.* (INPA 1249); 6 specimens,

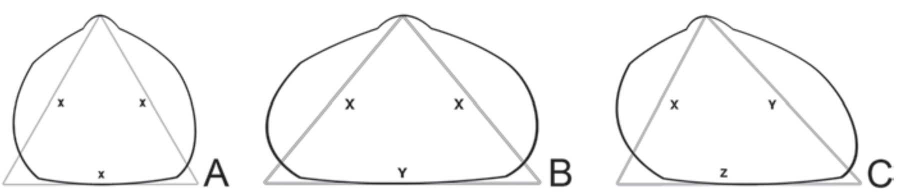
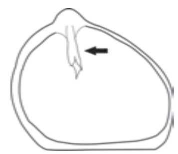


Figure 1. Schematic drawings of the three basic shapes of Amazonian glochidia, based on triangles. **A**, Equilateral, with all three sides approximately equal. **B**, Isosceles, with two sides of equal length and a generally longer dorsal line. **C**, Scalene, with all three sides of unequal lengths and the base edge displaced from the center. Letters represent hypothetical measurements and sides with the same letter are of the same length.

Table 1. Terms used to describe characteristics of glochidia of the Unionoida. **ES**, Spanish; **PT**, Portuguese.

Term adopted	Definition	Terms found in the literature / author	Schematic drawing	Taxa having the characteristic
Base edge	Ventral projection of the glochidia	Ventral edge: Clarke 1981		*Diplodon* (*Diplodon*), *Prisodon*, *Triplodon*, *Castalia*
Cusps	Sharp terminations of the glochidial hooks. A central, more elongated one directed towards the interior and two short lateral ones on the sinuous hook; a single sharp cusp or in the shape of a beak in the triangular hook.	Cúspides (PT): Mansur 1999, Mansur and Campos-Velho 1990 Cusps: Mansur and Silva 1999 Espinas (ES): Bonetto *et al.* 1986, Martínez 1983, Martínez and Royero 1995 Espinhos (PT): Mansur and Campos-Velho 1990 Espínulas (ES): Bonetto 1960b, Bonetto and Ezcurra 1965, Bonetto *et al.* 1986, Martínez and Royero 1995 Lancetas (ES): Bonetto 1960b, Martínez 1983 Lancets or microstylets: Mansur and Campos-Velho 2000 Punta aguda (ES): Martínez 1983		Unionidae, *Diplodon* (*Diplodon*), *Prisodon*, *Triplodon*, *Castalia*
Hook	Distal projection close to the ventral extremity and directed towards the inside of the glochidial shell. Triangular shape or elongate with wide base.	Dente (PT): Mansur 1999, Mansur and Campos-Velho 1990 Diente (ES): Bonetto 1954, 1955, 1961, Bonetto 1960b, Bonetto *et al.* 1986, Bonetto and Ezcurra 1965, Martínez 1983, Martínez and Royero 1995 Hook: Ortmann 1921, Kinzelbach and Nagel 1986, Jupiter and Byrne 1997, Wächtler *et al.* 2001, Vale *et al.* 2005 Spine: Mansur and Campos-Velho 2000 Stylet: Clarke 1985 Tooth: Mansur and Silva 1999		Unionidae, *Diplodon* (*Diplodon*), *Prisodon*, *Triplodon*, *Castalia*
Microstylets	Small projections in the form of spines found on the base and on the surface of the hooks.	Microstylet: Clarke 1985 Spines: Kinzelbach and Nagel 1986, Jupiter and Byrne 1997 Spinules: Ortmann 1921 Tooth: Wächtler *et al.* 2001		Unionidae
Protuberance	Rounded projection originating from the base of the hook.	Prominence: Mansur and Silva 1999 Protuberance: Vale *et al.* 2005 Tooth: Jupiter and Byrne 1997		*Diplodon* (*Diplodon*), *Castalia*, Prisodontini
Sinuous hook	Elongate projection, in the shape of an "S" viewed from the front of the valve, with three terminal cusps. In lateral view, the hook has the shape of an anchor, with a wide base* (arrow).	Dente em forma de S (PT): Mansur 1999, Mansur and Campos-Velho 1990 Diente en forma de S (ES): Bonetto *et al.* 1986 Diente en forma sigmoidea (ES): Bonetto and Ezcurra 1965 S-shaped tooth: Mansur and Silva 1999, Mansur and Campos-Velho 1990		*Diplodon* (*Diplodon*), *Prisodon*, *Triplodon*
Triangular hook	Triangular projection with wide base and a single cusp on the distal extremity. Cusp sharp or in the shape of a beak.	Dente triangular: Mansur and Campos-Velho 1990 Diente agudo (ES): Martínez 1983 Diente triangular (ES): Bonetto *et al.* 1986 Triangular hook: Vale *et al.* 2005 Triangular spine: Mansur and Campos-Velho 2000		Unionidae, *Castalia*

* Wu *et al.* (1999) denominate as "wings" what is here called the base.

05.VII.2007, D. M Pimpão *leg.* (INPA 1234); PERU, **Ucayali**: Pucallpa (Quebrada Durand, 08°23′S 74°33′W), 1 specimen, 13.IX.1996, K. Valdivia *leg.* (INPA 1363).

Triplodon corrugatus. BRAZIL, **Roraima**: (Branco River, 01°02′S 61°51′W) 9 specimens, 04.VII.2003, N. L. Chao *et al. leg.* (INPA 1074); **Pará**: Santarém (Alter do Chão, Tapajós River, 02°30′27.2″S 54°57′54.8″W) 1 specimen, 12.XI.2006, D. M Pimpão *et al. leg.* (INPA 1183).

Paxyodon syrmatophorus. BRAZIL, **Pará**: Santarém (Alter do Chão, Tapajós River, 02°30′41.2″S 54°57′59.3″W) 6 specimens, 03.X.2007, D. M Pimpão *leg.* (INPA 1355); (02°30′S 55°00′W), 1 specimen, 16.XI.1992, C. C. Fernandes *leg.* (INPA 1432).

Prisodon obliquus. BRAZIL, **Amazonas**: Novo Aripuanã (Aripuanã River, Pernambuco Beach, 06°01′05.1″S 60°11′39.5″W) 19 specimens, 06.IX.2007, D. M Pimpão *et al. leg.* (INPA 1264); 8 specimens, 07.IX.2007, D. M Pimpão and M. S. Rocha *leg.* (INPA 1288).

Castalia ambigua. BRAZIL, **Pará**: Irituia (Irituia River, in front of Irituia town 01°46′11.1″S 47°26′5.9″W - Station B, and 2 km downstream of Irituia town - Station A), 19–21.V.2001, R. Souza do Vale *leg.* (UFPA Bragança, no number).

RESULTS

Description of the glochidia

BIVALVIA
HETEROCONCHIA
HYRIIDAE
Glochidium type larvae with or without hooks

Diplodontini Parodiz and Bonetto, 1963
Diplodon (*Diplodon*) Spix, 1827
Glochidium with hook in the form of an "S" and protuberance present.

Diplodon (*Diplodon*) *suavidicus* (Lea, 1856)

Outline similar to a scalene triangle (Figs. 2A, 4A), length (ave. = 291.4 µm) greater than height (ave. = 246.5 µm); base edge pointed and conspicuous, displaced from the center (mean angle of obliquity = 14.7°); valves inequilateral, anterior margin greater and more arched than posterior; external and internal surfaces presenting subtle relief formed by microdepressions and elevations, similar to the surface of an egg-shell, without any apparent orientation; radial grooves and pores may be seen (diameter of 1 to 2 µm) in the center of the depressions of the external surface that cross the valves and are visible on the apex of elevations on the internal surface; valve border without pores, nearly smooth, presenting microscopic delicate perpendicular folds close to the margin; seen from the side, hook in the form of an anchor and with a

Table 2. Comparison of morphometric variables of the glochidia of seven species of Hyriidae from the Amazon. **Â**, angle of obliquity; **Ave.**, average; **CV**, coefficient of variation, **L**, length, **H**, height, **DLL**, dorsal line length, **SD**, standard deviation. All variables expressed in µm except for Â, which is expressed in degrees (°).

	L				H				DLL				Â			
Species	Max/mín	Ave.	*SD*	CV (%)	Max/mín	Ave.	*SD*	CV (%)	Max/mín	Ave.	*SD*	CV (%)	Max/mín	Ave.	*SD*	CV (%)
Triplodon corrugatus	298.7 251.9	273.3	11.4	0.04	260.8 217.0	237.7	9.09	0.04	210.7 154.9	179.2	11.32	0.06	16 7.5	12.0	1.73	0.14
Paxyodon syrmatophorus	254.5 223.0	242.5	5.33	0.02	198.9 167.6	187.0	5.13	0.03	194.6 151.3	178.9	6.79	0.04	7.0 0	3.5	1.33	0.38
Prisodon obliquus	251.4 232.1	241.5	5.18	0.02	182.2 164.8	174.1	4.37	0.02	202.0 168.6	186.7	7.62	0.04	7.0 1.0	4.7	1.64	0.35
Castalia ambigua	282.0 251.9	262.7	6.0	0.02	271.9 246.6	258.5	5.15	0.02	192.9 166.8	181.3	5.93	0.03	6.0 0	2.2	1.58	0.73
Diplodon hylaeus	312.9 275.4	293.6	7.07	0.02	252.2 222.7	238.8	5.87	0.02	225.2 169.0	194.8	10.52	0.05	21.0 12.0	16.3	1.64	0.10
Diplodon suavidicus	302.4 279.6	291.4	5.16	0.02	266.2 229.3	246.5	6.75	0.03	220.6 181.6	204.2	8.01	0.04	18.5 11.5	14.7	1.37	0.09
Diplodon obsolescens	334.9 303.3	318.8	9.17	0.03	280.6 252.4	265.4	10.05	0.04	234.0 193.3	216.7	13.21	0.06	12.0 6.0	8.1	1.77	0.22

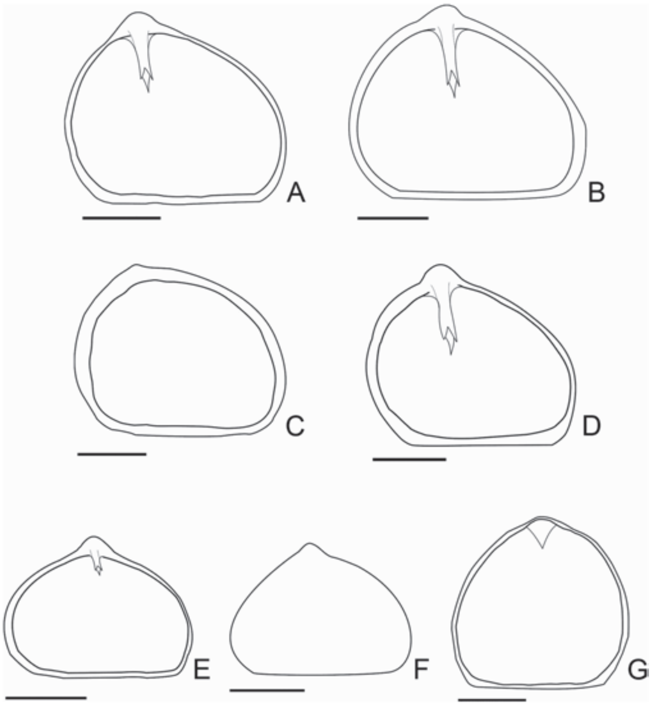


Figure 2. Outlines of glochidia of Amazonian Hyriidae. **A**, *Diplodon suavidicus*. **B**, *Diplodon obsolescens*. **C**, *Diplodon hylaeus*, a hookless glochidium. **D**, *Triplodon corrugatus*. **E**, *Paxyodon syrmatophorus*. **F**, *Prisodon obliquus*, incompletely formed glochidium, as yet without a complete hook. **G**, *Castalia ambigua* Scale bar = 100 µm.

wide triangular base, which narrows gradually and straightens in the center; three terminal cusps occur at the distal extremity, two are smaller, one on each side, and a longer sharp central cusp, pointing towards the inside of the valve, with the distal extremity directed towards the dorsal line; seen from the front, the hook has an "S" shape (Figs. 3A, 4B–4C), the hook is open, in the form of a groove, on the internal face, which allows the opposite hook to fit when the valves close; hook length (ave. = 90.6 µm) approximately one and a half to two times the width of the base; triangular protuberance with rounded apex, projected between the ventral base of the hook and the base edge as though a prolongation of itself, directed towards the inside of the valve, sometimes absent with a foramen in its place. Usually a more robust protuberance seen on one valve and a foramen on the other, sometimes with a small protuberance. Gravid females were collected in August (falling to low water period in the Jamari River, Roraima state) and September (falling water period ["*descida das águas*"] in the middle to lower reaches of the Aripuanã River, Amazonas state).

***Diplodon* (*Diplodon*) *obsolescens* F. Baker, 1914**

Outline similar to a scalene triangle (Figs. 2B, 4D), length (ave. = 318.8 µm) greater than height (ave. = 265.4 µm); base edge pointed and displaced from the center (mean angle of obliquity = 8.1°), valves inequilateral, anterior margin taller and more arched than posterior. External and internal surfaces presenting subtle relief formed by microdepressions and elevations, similar to the surface of an eggshell, with apparent commarginal orientation; presence of radial grooves and pores (diameter of 1 to 2 µm) in the center of the depressions of the external surface that cross the valves and are visible on the apex of elevations on the internal surface; larger pores are concentrated in the central part of the valves along with the adductor muscle scar; valve border without pores and apparently smooth, with microscopic delicate perpendicular folds close to the margin. Seen from the side, hook in the form of an anchor, with a wide triangular base, the margins of which are concave on immediately projecting from the base (border in this case), after which they proceed straight towards the distal end of the hook, which has three terminal cusps, two are smaller, one on each side, and a longer central sharp cusp directed towards the inside of the valve, with the distal extremity directed towards the dorsal line. Seen from the front of the glochidium, the hook has an "S" shape (Figs. 3B–3C, 4E); on the internal face the hook is open, in the form of a groove, which allows the opposite hook to fit, when the valves close. Hook length (ave. = 114.1 µm) approximately twice the width of the base; protuberance projected from the ventral base of the hook, as a continuation of the base edge, with the distal edge rounded or flattened, sometimes a foramen is present. Gravid females were collected in July (falling water period on the upper Aripuanã River, Mato Grosso state).

Diplodon (*Rhipidodonta*) Mörch, 1853
Glochidium without either hook or protuberance.

***Diplodon* (*Rhipidodonta*) *hylaeus* (d'Orbigny, 1835)**

Outline similar to a scalene triangle (Figs. 2C, 4F), length (ave. = 293.6 µm) greater than height (ave. = 238.8 µm), base edge rounded, without a projection, displaced from the center (mean angle of obliquity = 16.3°). Valves strongly inequilateral, anterior margin almost as convex as posterior. Pores that pass through valves visible on the internal (Fig. 3D) and external surfaces. Glochidium without either a hook or a protuberance. External surface with a very evident relief formed by deep elevations and depressions with apparent commarginal orientation; these elevations present a rounded apex and the sides are grooved, the grooves (numbering 3 to 5) converge to the pores in the center of the depressions, the structure is reminiscent of upholstered material, but without a regular pattern. Some of these grooves also advance over the borders of the valve, which do not present pores, forming regularly spaced groups of divergent radial microfurrows/grooves; internally

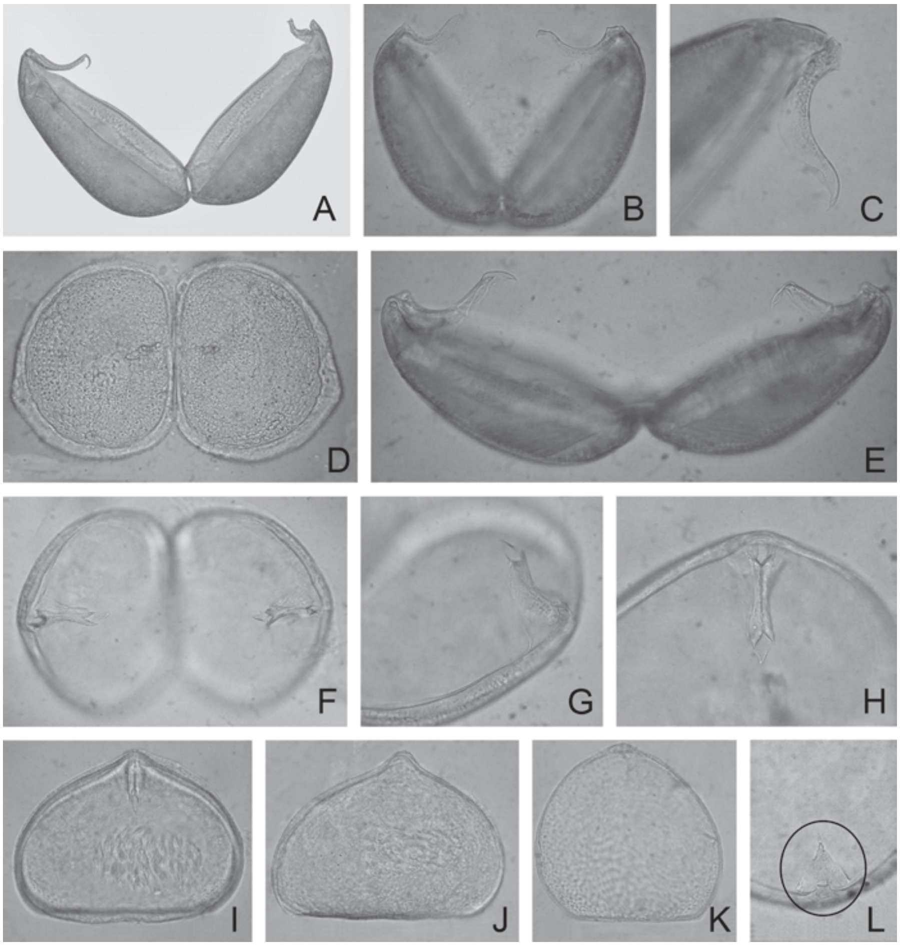


Figure 3. Photomicroscopy of the glochidia of Amazonian Hyriidae. **A**, *Diplodon suavidicus*, frontal view of valves with profile of hook. **B–C**, *Diplodon obsolescens*. **B**, Frontal view of valves. **C**, Profile of hook. **D**, *Diplodon hylaeus*, lateral view, valves open. **E–H**, *Triplodon corrugatus*. **E**, Frontal view of the valve. **F**, Ventral view. **G**, Hook in latero-ventral view. **H**, Ventral view of hook. **I**, *Paxyodon syrmatophorus*, lateral view of valve. **J**, *Prisodon obliquus*, lateral view of valve. **K–L**, *Castalia ambigua*. **K**, Lateral view of valve. **L**, Ventral view of hook. Magnification 400 X.

the border of the valves is smooth, wide and very much detached from the rest of the internal surface (Fig. 4G) that presents rounded elevations topped by pores. Gravid females were collected in July (falling water period on the upper Aripuanã River, Mato Grosso state).

Prisodontini Modell, 1942

Triplodon Spix, 1827

Glochidium with a hook in the shape of an "S" and presence of a protuberance; characteristics similar to those of species of *Diplodon* (*Diplodon*).

***Triplodon corrugatus* (Lamarck, 1819)**

Outline similar to a scalene triangle (Figs. 2D, 4H), length (ave. = 273.3 μm) greater than height (ave. = 237.7 μm), base edge pointed and displaced from the center (mean angle of obliquity = 12.0°); valves inequilateral, anterior margin taller and more arched than posterior; pores that cross the valves visible on the internal and external surfaces. External surface with a very delicate relief consisting of chains of rounded depressions with a central pore, forming a fine and densely dotted pattern that is reminiscent of an eggshell surface, with an apparent commarginal orientation; valve border filled with more or less parallel microfolds, perpendicular to the border, also present on the protuberance and on the hook (Fig. 4I); reinforced hook, with a very wide base; in the form of an anchor in lateral view (Figs. 3F, 3H), narrows toward distal end and proceeds straight along the central part, as it approaches the three terminal cusps: one central, elongate and projected towards the dorsal line of the valve, and two small, short laterals (Fig. 4J); seen from the front the hook has an "S" shape (Figs. 3E, 3G); the internal face is open as in species of *Diplodon* (*Diplodon*) described above; hook length (ave. = 102.5 μm) approximately one and a half times the width of the base; protuberance projected from the ventral base of the hook, with rounded extremity. Gravid females collected in November (falling to low water period in the lower Tapajós River, Pará state), July (high water on the lower Branco River, Roraima state) and May (end of high water period on the Pará River, Melgaço, Pará state).

Paxyodon Schumacher, 1817

Glochidium with short and straight to slightly sinuous hook, protuberance present; external surface with microprojections in the form of spines.

***Paxyodon syrmatophorus* (Meuschen, 1781)**

Outline similar to an isosceles triangle (Figs. 2E, 4K), length (ave. = 242.5 μm) greater than height (ave. = 187.0 μm); base edge pointed and slightly displaced from the center (mean angle of obliquity = 3.5°); valves nearly equilateral (Fig. 3I), anterior margin slightly taller and more arched than posterior. External surface presenting shallow depressions and subtle elevations, reminiscent of a slightly hammered

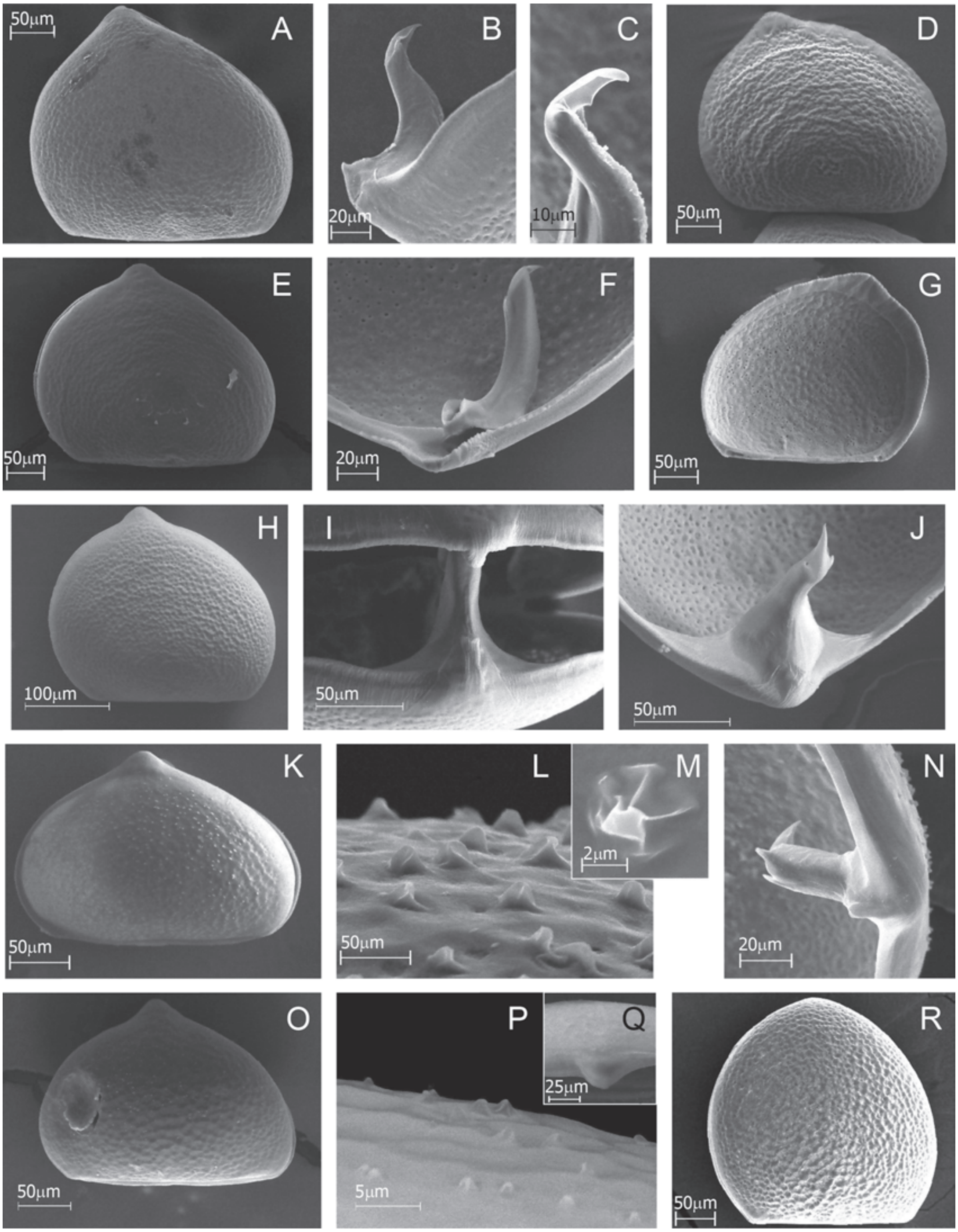


Figure 4. Photomicrography of the glochidia of Amazonian Hyriidae. **A–C**, *Diplodon suavidicus*. **A**, Lateral view of the valve. **B–C**, Hook in detail. **D–E**, *Diplodon obsolescens*. **D**, Lateral view of the valve. **E**, Internal-ventral view of hook. **F–G**, *Diplodon hylaeus*, valves. **F**, External lateral view. **G**, Internal view. **H–J**, *Triplodon corrugatus*. **H**, Lateral view of the valve. **I**, United hooks and protuberance at ventral basis of hook. **J**, Ventral view of the hook. **K–N**, *Paxyodon syrmatophorus*. **K**, Lateral view of the valve. **L**, Detail of the external surface with spikes. **M**, Detail of a spike. **N**, Ventral view of the hook. **O–Q**, *Prisodon obliquus*. **O**, Lateral view of the valve. **P**, External surface in detail with spikes. **Q**, Detail of hook formation. **R**, *Castalia ambigua*, lateral view of the valve.

surface; depressions with one or two pores that cross the valves; presence of microprojections as spikes (Fig. 4L) with a rounded distal extremity and a palmate base, whose extremities form structures similar to anchors (Fig. 4M); basal projections of spikes generally number three to five; valve border with perpendicular microfolds, less numerous and wider on the protuberance; microfolds also present on the base of the hook; at high magnifications microgranules may be observed on the borders and on the hook. Hook in the form of an anchor in lateral view, with a wide triangular base that tapers gradually towards the central region, relatively short, three cusps on the distal extremity, two smaller ones, one on each side, and a longer sharp central cusp, projected towards the dorsal line (Fig. 4N); hook length (ave. = 62.8 μm) approximately equal to the width of the base; hook almost straight seen from the front, slightly inclined inwards (groove not seen); protuberance projected from the ventral base of the hook (Fig. 4N), with rounded distal extremity. Gravid females collected in October (falling water period on the lower Tapajós River, Pará state) and May (end of high water period on the Pará River, Melgaço, Pará state).

Prisodon Schumacher, 1817

Glochidium with a hook, external surface with microprojections in the form of spikes.

***Prisodon obliquus* Schumacher, 1817**

Outline similar to an isosceles triangle (Figs. 2F, 3J, 4O), length (ave. = 241.5 μm) greater than height (ave. = 174.1 μm); base edge pointed in the center (half the valve length) or slightly displaced from the center (mean angle of obliquity = 4.7°); valves nearly equilateral, anterior and posterior margin equally convex. External surface with a very subtle relief of depressions and elevations forming a hammered pattern; pores (one to two) that cross the valves, visible on the internal and external surfaces; microprojections similar to spikes (Fig. 4P) with a rounded distal extremity and a palmate base; basal projections of spikes generally number from three to five; as in *Paxyodon syrmatophorus*; borders with perpendicular microfolds and punctuations. The glochidia obtained presented an externally complete and well-formed shell, the hooks present were, however, still being formed. The formation of the protuberance and the outline of the hook were observed in an incomplete specimen with slightly deflected borders (Fig. 4Q). Gravid females were collected in September (falling water period in the middle to lower Aripuanã River, Amazonas state).

Castaliini Parodiz and Bonetto, 1963

Castalia Lamarck, 1819

Glochidium with triangular hook slightly curved with beak, protuberance present.

***Castalia ambigua* (Lamarck, 1819)**

Outline similar to an equilateral triangle (Figs. 2G, 3K, 4R), length (ave. = 262.7 μm) similar to height (ave. = 258.5 μm); base edge pointed in the center (mean angle of obliquity = 2.2°). Valves nearly equilateral, anterior and posterior margins equally convex. External surface with a relief formed by rows of depressions and elevations with an apparently commarginal orientation similar to a hammered pattern; depressions with pores (from one to three) that cross the valves. Valve border without pores and apparently smooth, however, at high magnifications, delicate perpendicular folds may be seen that anastomose in an irregular fashion. Triangular hook uniform with a wide base in lateroventral view (Fig. 3L), lateral margins almost straight and convergent on the distal extremity; in frontal view, the hook is curved inwards resembling a beak; hook length (ave. = 43.7 μm) approximately equal to the width of the base; a single cusp on the rounded or flattened distal extremity. Protuberance and hook with occasional microfolds similar to those at the borders. Gravid females were collected in May (end of high water period on the Irituia River, Pará state). (In part Vale *et al.* 2005).

Multivariate analysis

Canonical Discriminant Analysis

Glochidia of each genus were distinguished by CDA. Of the total variability, 82.2% is explained by Function 1 in which height is the principal variable responsible (0.903). Function 2 represents 14.8% of the variation, influenced by the angle of obliquity (0.613).

CDA resulted in the formation of three groups (Fig. 5): the first is composed of *Prisodon obliquus* and *Paxyodon syrmatophorus*; the second includes *Triplodon corrugatus* and the three species of *Diplodon*; and the third representing only *Castalia ambigua*. Although the glochidia of *Prisodon obliquus* and *Paxyodon syrmatophorus* were most similar between themselves than in comparison with those of the other species, both were clearly distinguished in the analysis (see centroid, Fig. 5). The *Prisodon*-*Paxyodon* group was separated from the others by the height of the glochidial shell (Function 1). *Triplodon corrugatus* was more similar to the three species of *Diplodon*. However, these four species were also clearly distinguished in the analyses (see centroids). Finally, *Castalia ambigua* was the species that was most distinct from all the others in terms of the glochidial shell shape, as a result of the angle of obliquity (Function 2).

DISCUSSION

Both morphological descriptions and morphometric analyses were efficient in identifying and distinguishing among genera and species and in the classification of supra

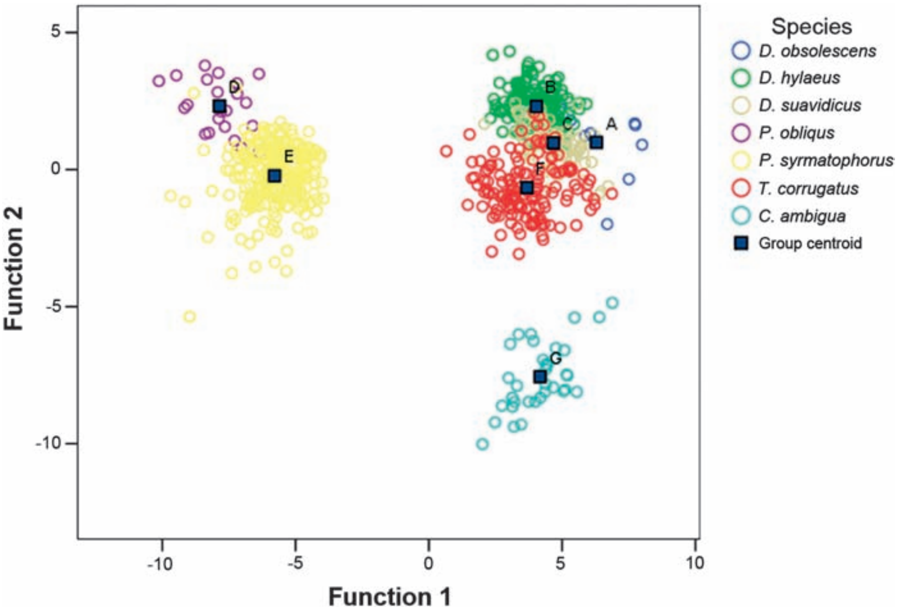


Figure 5. Canonical Discriminant Analysis of the morphometry of glochidial shells of Amazonian hyriid species: *Diplodon suavidicus*, *D. obsolescens*, *D. hylaeus*, *Prisodon obliquus*, *Paxyodon syrmatophorus*, *Triplodon corrugatus* and *Castalia ambigua*, showing three distinct groups of larvae.

specific taxa. The analysis separated the larvae into three groups that coincide with the three basic shapes of Amazonian glochidia of Hyriidae.

The presence of spikes, seen using SEM, on the external surface of glochidia of *Prisodon obliquus* and *Paxyodon syrmatophorus*, here described for the first time, but absent in other South American species (Martínez 1983, Martínez and Royero 1995, Mansur and Silva 1999, Mansur and Campos-Velho 2000, Vale *et al*. 2005), including *Triplodon*, constitutes a unique characteristic of *P. obliquus* and *P. syrmatophorus*, in terms of ultrastructural larval morphology. Based on the morphology of the hinge of adult shells, Olsson and Würtz (1951) grouped *P. obliquus* with the other member of the Prisodontini, *T. corrugatus*, both distinct from *Paxyodon syrmatophorus*. This classification was followed by Simone (2006). However, the similarity between *P. obliquus* and *P. syrmatophorus* is supported here by glochidium morphometrics, in which both species form a well defined and consistent group that may suggest their union under the genus *Prisodon*, distinct from *Triplodon*. A cladistic study (Pimpão 2010), using adult shell and soft parts, provides further evidence for affinities between *Prisodon* and *Paxyodon*.

Diplodon and *Triplodon* have, up to now, always been separated on the basis of adult shell characteristics alone. The presence of posterior and anterior alate projections on the shell is diagnostic of the Prisodontini, despite the fact that smaller specimens have reduced projections. Morphometric analysis and ultrastructural observations from the present study provide evidence for the morphological similarity of the larvae of *Diplodon* spp. and *Triplodon corrugatus*.

Glochidia belonging to the genus *Diplodon* were either hooked or hookless, a character that allowed the separation of one of the species, *D. hylaeus*. Ortmann (1921), Bonetto (1961b) and Haas (1969), also using the presence/absence of the glochidial hook, separated *Diplodon* into two subgenera: *Diplodon* (*Diplodon*) and *D.* (*Rhipidodonta*). In a survey of larvae of *Diplodon*, Bonetto (1961b) measured and described the glochidium of *D. suavidicus* as being non-parasitic. In the description the author informs that the larvae were from a single specimen from the upper Xingu River, Mato Grosso state. The hookless glochidia in the present study are from a nearby region (upper Aripuanã River) in the same state (Mato Grosso) and were identified as *Diplodon hylaeus.* Ortmann (1921) also mentions that this species [sic] (*D. hasemani*) has non-parasitic glochidia. Shells of adult *D. hylaeus* from the upper Aripuanã River have very worn umbos and faintly marked shell sculpture. This may possibly have caused Bonetto (1961b) to misidentify *D. hylaeus* from the upper Xingu as *D. suavidicus*, which has a similarly weak shell sculpture. Morphometric analysis show that both species overlap somewhat on the ordination, despite the centroids occupying separate positions.

The first author to question the validity of the species *Diplodon obsolescens* and *D. suavidicus* was Bonetto (1960, 1961b) who considered both as synonyms. Simone (2006) raised the subgenus *Rhipidodonta* to the level of genus and maintained the above mentioned Amazonian species as synonyms in the genus *Rhipidodonta*, despite the revalidation of *D. obsolescens* by Mansur and Valer (1992) on the basis of a comparison of morphological characteristics of the shells. Pimpão and Mansur (2009) also distinguished between *D. obsolescens* and *D. suavidicus* on the basis of adult shell characteristics. In the present study, glochidia of both species were found to be hooked. Since the presence of glochidial hooks is a diagnostic characteristic of the subgenus *Diplodon senso stricto*, it is suggested here that both species should be united in *Diplodon* (*Diplodon*). The smaller larval shell length of *D. suavidicus* (ave. = 291.4 μm) allows it to be distinguished from the larger glochidium of *D. obsolescens* (ave. = 318 μm). Subgenera of *Diplodon* are distinguished only according to the presence of glochidial tooth in *Diplodon* (*Diplodon*) or absence in *D.* (*Ripidodonta*). Simone (2006) erected the subgenus to a higher level (genus) without providing any diagnostic characters. Considering that glochidia of many Diplodotini species are still unknown, and that criteria based on adult shell and soft parts are lacking for *Rhipidodonta*, it was an option of these authors to maintain it at the subgeneric level, until new studies could evidence the differences between both genera.

In terms of shape, the glochidium of *Castalia ambigua* was distinct from those of all the other species due to differences in the angle of obliquity, which, according to Vale *et al.* (2005), the obliquity is close to zero. Other authors have also observed this striking characteristic of species of Castaliini (Bonetto 1961a, 1965, Martínez 1983).

CONCLUSION

The morphometric comparison of the outline, sculptural patterns, and hooks of glochidial shells provide further evidence for the maintenance of the two subgenera in *Diplodon* and, within the Prisodontini, indicate the separation of *Triplodon* from *Prisodon* and *Paxyodon*. The external surface sculpture ornamentation of the glochidia with the presence of spikes, seen only under SEM, and described for the first time, reinforces this separation.

The morphometric comparison, using multivariate analyses, allowed separation of the species of Hyriidae into three groups that coincide with the three basic shapes of Amazonian glochidia.

The larvae of *Castalia* show wide divergence in relation to the other genera, principally with respect to the angle of obliquity in relation to the base edge.

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